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Antimicrobial Susceptibility of *Brucella melitensis* Isolates in Peru[▽]

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Brucellosis is an important public health problem in Peru. We evaluated 48 human *Brucella melitensis* biotype 1 strains from Peru between 2000 and 2006. MICs of isolates to doxycycline, azithromycin, gentamicin, rifampin, ciprofloxacin, and trimethoprim-sulfamethoxazole were determined by the Etest method. All isolates were sensitive to tested drugs during the periods of testing. Relapses did not appear to be related to drug resistance.

Infection by *Brucella* species is a major cause of zoonotic disease. Brucellosis is endemic in the Mediterranean littoral, southwest and central Asia, the Indian subcontinent, and Latin America (12). In Peru, approximately 1,000 cases of human brucellosis (overwhelmingly due to *B. melitensis*) are reported per year; up to 25% of these cases are identified in Callao, the port city of the capital, Lima (11). Infection in Peru typically results from the consumption of unpasteurized dairy products or, less commonly, from occupational exposure to the products of conception of infected mammals (13). Ongoing vaccination campaigns directed at preventing goat brucellosis may be reducing the number of human cases (10).

Commonly recommended agents for the treatment of brucellosis include doxycycline (DOX), rifampin (RIF), streptomycin, gentamicin (GE), and trimethoprim-sulfamethoxazole (SXT); generally, two or three drugs are used in combination for 6 weeks or longer, depending on the location of infection and the associated clinical syndrome. Certain fluoroquinolones (e.g., levofloxacin) and macrolides (e.g., azithromycin [AZM]) may have an adjunctive role in the management of the disorder (2, 8, 14).

Antimicrobial drug resistance in *Brucella* is unusual. Increases in the MICs of ceftriaxone and streptomycin have been reported in Turkey (15), although these agents remain active. Intermediate rifampin susceptibility elsewhere in Turkey has been described previously (1). Limited *in vitro* susceptibility to rifampin and trimethoprim-sulfamethoxazole in Kuwait (5) and Mexico (9) has similarly been reported.

In this study, we sought to evaluate the susceptibility of *Brucella melitensis* from human clinical blood cultures in Lima and Callao, Peru, to common antimicrobial drugs. We additionally wished to determine any changes in susceptibility during two distinct time periods in this area of endemicity. Lastly, we intended to examine whether *Brucella* isolates obtained from patients with relapsed disease differed in terms of susceptibility from specimens obtained during primary infections.

(The data included in this paper were presented in part at the 110th General Meeting of the American Society for Microbiology, San Diego, CA, 20 May 2010.)

The study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable U.S. federal regulations governing the protection of human subjects. We identified 48 *B. melitensis* isolates from human clinical blood culture specimens. Twenty-five isolates were obtained between January 2000 and April 2001 from patients hospitalized at the Centro Médico Naval (Callao, Peru) or the Hospital Arzobispo Loayza (Lima, Peru). An additional 23 isolates were obtained between September 2005 and May 2006 from patients hospitalized at the Hospital Nacional Daniel Alcides Carrión (Callao, Peru).

All cultures, species identification, and antimicrobial susceptibility tests were performed in the Bacteriology Laboratory of the Naval Medical Research Center Detachment (NMRCD) using the Ruiz-Castañeda and lysis centrifugation methods as previously described (3, 6, 7). Briefly, suspected colonies were identified on *Brucella* agar supplemented with 5% sheep's blood and then evaluated based upon Gram stain appearance, growth characteristics, and biochemical testing. Specimens were confirmed by slide agglutination using anti-*Brucella* polyclonal serum. Further determination of species and biotype was conducted by testing CO_2 growth requirements, urease and H_2S production, dye sensitivity using thionine and basic fuchsin, and agglutination with monospecific antisera to A and M antigens.

Antimicrobial susceptibility testing on confirmed *B. melitensis* isolates was then conducted using the Etest method (AB bioMérieux, Solna, Sweden). Mean MICs of DOX, AZM, GE, RIF, ciprofloxacin (CIP), and SXT were tested by inoculating a suspension of bacteria (adjusted to 0.5 McFarland units) onto Mueller-Hinton agar plates sup-

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TABLE 1. Antimicrobial susceptibility of 48 *Brucella melitensis* isolates[a]

Antibiotic	2000–2001			2005–2006			P^b	Breakpoint[c] for susceptibility (mg/liter)
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range		
Doxycycline	0.19	0.38	0.064–0.5	0.38	0.38	0.032–0.38	0.022	≤2
Azithromycin	0.25	0.5	0.064–0.5	0.25	0.50	0.064–0.50	0.310	≤4
Gentamicin	0.125	0.25	0.064–0.250	0.125	0.226	0.032–0.25	0.029	ND[d]
Rifampin	0.50	0.75	0.380–1.0	0.38	0.50	0.19–0.50	<0.0001	≤1
Ciprofloxacin	0.125	0.214	0.064–0.250	0.125	0.25	0.094–0.25	0.013	≤1
Trimethoprim-sulfamethoxazole	0.064	0.151	0.012–0.64	0.032	0.094	0.016–0.125	0.134	≤0.5

[a] Isolates were from the Centro Médico Naval and Hospital Arzobispo Loayza (January 2000 to April 2001) and Hospital Nacional Daniel Alcides Carrión (September 2005 to May 2006). All MICs are in mg/liter.
[b] *P* values reflect the comparison between MIC_{50} values over the two time periods (or MIC_{90}, in the case of doxycycline).
[c] Standard breakpoints are from CLSI guidelines for slowly growing bacteria (*Haemophilus* spp.) (4).
[d] ND, not defined by CLSI standards.

plemented with 5% sheep's blood, followed by the application of Etest strips. MICs were then determined following 48 h of incubation.

The following reference strains were used for quality control during susceptibility testing: *Escherichia coli* 25922, *Staphylococcus aureus* 25923, *Brucella abortus* 2308, *Brucella melitensis* rev-1, and *Brucella canis* RM6/66. All reference strains were obtained from the American Type Culture Collection, Manassas, VA. We interpreted MIC values according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for slow-growing bacteria (*Haemophilus* spp.) as described elsewhere (4). Statistical significance was determined by analysis of variance (ANOVA) using SPSS version 17.0 (SPSS Inc., Chicago, IL).

All 48 isolates were confirmed as *B. melitensis* and agglutinated with anti-M monospecific sera, consistent with biotype 1. Five of the 48 isolates were from patients with relapsed disease, while the remaining 43 were from primary infections. The MIC_{50} and MIC_{90} values of tested antibiotics are shown in Table 1. All isolates were generally susceptible to the tested agents. One isolate had reduced susceptibility to RIF (MIC: 1.0 mg/liter) and one to SXT (MIC: 0.64 mg/liter).

Increased antimicrobial resistance did not appear to develop over the two tested periods. In particular, strains from 2000 to 2001 had higher mean rifampin MIC_{90} values than those obtained in 2005 to 2006 (0.75 mg/liter versus 0.50 mg/liter). Similarly, there were small increases in the mean doxycycline and ciprofloxacin MIC_{50}s and a small decrease in gentamicin MIC_{90} (Table 1). Although the changes in mean doxycycline, gentamicin, rifampin, and ciprofloxacin MICs between the two periods were statistically significant, the absolute differences were small and did not appear clinically meaningful.

Isolates from patients with relapsed disease were compared with primary specimens from the same patients when available. Three of five patients had primary specimens available. There were no observed differences in the antimicrobial susceptibilities of strains from patients with primary infection versus those from patients in relapse. No resistance was detected in the isolates from patients in relapse, and MIC values were generally low (Table 2).

In summary, *Brucella melitensis* strains causing human disease with bacteremia in metropolitan Lima, Peru, are broadly susceptible to common antibiotics, with no new resistance noted in the periods of time studied. Relapse of clinical disease does not appear to be associated with antimicrobial drug resistance. These results, however, are limited by a narrow geographic scope and relatively small numbers. The routine evaluation of drug susceptibility in *Brucella* species is hampered by the lack of *Brucella*-specific CLSI guidelines, the need for biosafety level 3 conditions during susceptibility testing, and the risk of laboratory staff exposure. Periodic testing of a subset of isolates in reference laboratories may be a more appropriate and safer means for monitoring *Brucella* drug susceptibility over broad regions.

TABLE 2. MIC_{50} values of selected antimicrobial drugs for patients with relapsed brucellosis[a]

Patient	Mo after primary infection	MIC (mg/liter) of:					
		DOX	CIP	SXT	GE	RIF	AZM
1	0	0.250	0.094	0.064	0.190	0.500	0.250
	3	0.250	0.125	0.094	0.125	0.500	0.500
2	0	0.125	0.125	0.032	0.250	0.500	0.380
	3	0.190	0.125	0.016	0.125	0.500	0.380
3	0	0.250	0.125	0.064	0.190	0.500	0.500
	6	0.125	0.094	0.016	0.190	0.500	0.380
4	3	0.19	0.094	0.064	0.032	0.125	0.38
5	6	0.094	0.125	0.032	0.19	0.25	0.5

[a] Initial specimens were available for patients 1, 2, and 3 only.

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REFERENCES

1. **Aliskan, H., T. Turunc, Y. Z. Demiroglu, S. Colakoglu, and H. Arslan.** 2008. Investigation of in vitro antibiotic susceptibility of Brucella melitensis. Mikrobiyol. Bul. **42:**125–129.
2. **Ariza, J., et al.** 2007. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med. **4:**e317.
3. **Castaneda, M. R.** 1947. A practical method for routine blood cultures in brucellosis. Proc. Soc. Exp. Biol. Med. **64:**114.
4. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing, vol. 29; 19th international supplement. M100-S19-MIC. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Dimitrov, T., D. Panigrahi, M. Emara, F. Awni, and R. Passadilla.** 2004. Seroepidemiological and microbiological study of brucellosis in Kuwait. Med. Princ. Pract. **13:**215–219.
6. **Espinosa, B. J., et al.** 2009. Comparison of culture techniques at different stages of brucellosis. Am. J. Trop. Med. Hyg. **80:**625–627.
7. **Etemadi, H., A. Raissadat, M. J. Pickett, Y. Zafari, and P. Vahedifar.** 1984. Isolation of Brucella spp. from clinical specimens. J. Clin. Microbiol. **20:**586.
8. **Falagas, M. E., and I. A. Bliziotis.** 2006. Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrob. Agents Chemother. **50:**22–33.
9. **Lopez-Merino, A., et al.** 2004. Susceptibility of Mexican brucella isolates to moxifloxacin, ciprofloxacin and other antimicrobials used in the treatment of human brucellosis. Scand. J. Infect. Dis. **36:**636–638.
10. **Mendoza-Nunez, M., et al.** 2008. Brucellosis in household members of Brucella patients residing in a large urban setting in Peru. Am. J. Trop. Med. Hyg. **78:**595–598.
11. **Nockler, K., et al.** 2009. Molecular epidemiology of Brucella genotypes in patients at a major hospital in central Peru. J. Clin. Microbiol. **47:**3147–3155.
12. **Pappas, G., N. Akritidis, M. Bosilkovski, and E. Tsianos.** 2005. Brucellosis. N. Engl. J. Med. **352:**2325–2336.
13. **Smits, H. L., et al.** 2009. MLVA genotyping of human Brucella isolates from Peru. Trans. R. Soc. Trop. Med. Hyg. **103:**399–402.
14. **Solera, J., J. L. Beato, E. Martinez-Alfaro, J. C. Segura, and E. de Tomas.** 2001. Azithromycin and gentamicin therapy for the treatment of humans with brucellosis. Clin. Infect. Dis. **32:**506–509.
15. **Tanyel, E., et al.** 2007. Actual antibiotic resistance pattern of Brucella melitensis in central Anatolia. An update from an endemic region. Saudi Med. J. **28:**1239–1242.